# Light and scanning electron microscopic evaluation of Glyde$^{TM}$ File Prep in smear layer removal

**T. S. Lim, T. Y. Wee, M. Y. Choi, W. C. Koh & V. Sae-Lim**
Department of Restorative Dentistry, Faculty of Dentistry, National University of Singapore, Republic of Singapore

## Abstract

**Lim TS, Wee TY, Choi MY, Koh WC, Sae-Lim V.** Light and scanning electron microscopic evaluation of Glyde$^{TM}$ File Prep in smear layer removal. *International Endodontic Journal*, **36**, 336–343, 2003.

**Aim** To evaluate the effectiveness of Glyde$^{TM}$ File Prep used in conjunction with sodium hypochlorite irrigation in the removal of smear layer produced during root canal instrumentation.

**Methodology** Thirty-nine extracted human teeth with single root canals were used. Access cavities were prepared and the teeth divided into three groups of 13 teeth each. Each group was treated by one of the three different regimes of irrigation and conditioning during root canal instrumentation. Group A: 0.5 mL of 1% NaOCl irrigation after each file size with an additional final irrigation of 10 mL 1% NaOCl; group B: 0.5 mL of 1% NaOCl irrigation after each file size with an additional final irrigation of 10 mL 17% EDTA; group C: Glyde$^{TM}$ File Prep coated on each instrumentation file used in conjunction with 0.5 mL 1% NaOCl irrigation after each file size and an additional final irrigation of 10 mL 1% NaOCl. The teeth were then longitudinally grooved and sectioned. Root canal cleanliness was evaluated with the aid of a Nikon light microscope (×40 and ×100) and scanning electron microscope (×1000 and ×3000). The debris scores obtained at three canal regions were compared statistically within the same group and among different groups using repeated measurements of analysis of variance (ANOVA) with Bonferroni adjustments and ANOVA with posthoc Tukey HSD, respectively.

**Results** The canals treated with EDTA and Glyde$^{TM}$ File Prep were significantly cleaner than those treated with NaOCl alone. The apical region of the root canals generally displayed more residual smear layer, but the difference was not significant.

**Conclusions** Used in conjunction with NaOCl irrigation, Glyde$^{TM}$ File Prep was effective in removing smear layer produced during root canal instrumentation.

**Keywords:** canal cleanliness, root canal conditioner, smear layer.

*Received 22 April 2002; accepted 21 November 2002*

## Introduction

Successful root canal treatment depends primarily on the removal of microorganisms through chemo-mechanical instrumentation of the root canal system. This encompasses shaping by mechanical removal of the dentine and cleaning by chemical disinfection of microorganisms and dissolution of organic tissues from the root canal (Grossman 1988). While root canal shaping can be predictably and efficiently attained with advanced instrumentation technology, effective cleaning of the entire root canal system remains a challenge.

Sodium hypochlorite (NaOCl) irrigant used at varying concentration ranging from 0.5 to 5.25% has been widely accepted as an adjunct to improve cleaning due to its antiseptic property and organic tissue-dissolving capability (Moorer & Wesselink 1982). Nevertheless, it has been observed that NaOCl alone is ineffective in the removal of the smear layer that consists of organic and inorganic debris, formed on the root canal wall and/or occluding the dentinal tubular openings after instrumentation (McComb & Smith 1975, Goldman *et al.* 1981, Mader *et al.* 1984). Though the influence of smear layer

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Correspondence: Dr Varawan Sae-Lim, Department of Restorative Dentistry, Faculty of Dentistry, National University of Singapore, 5 Lower Kent Ridge Road, Singapore 119074, Republic of Singapore (Tel.: +65 6 7724954; fax: +65 6 7732603; e-mail: rsdvsl@nus.edu.sg).

on outcome has yet to be ascertained, it is considered to be desirable to remove the smear layer due to its potential deleterious effects. For example, the smear layer might harbour microorganisms (Baker *et al.* 1975, Yamada *et al.* 1983) and infect dentinal tubules (Meryon *et al.* 1987, Uitto *et al.* 1988, Meryon & Brook 1990), impede penetration or diffusion of antibacterial irrigants and medications into the dentinal tubules (Goldberg & Abramovich 1977, Bystrom & Sundqvist 1986, Baumgartner & Mader 1987), as well as compromise the seal between the filling materials and dentinal wall (Lester & Boyde 1977, Mader *et al.* 1984, White *et al.* 1984, Kennedy *et al.* 1986, Cergneux *et al.* 1987, White *et al.* 1987, Saunders *et al.* 1992, Oksan *et al.* 1993). The combined use of NaOCl in conjunction with other irrigating agents has thus been investigated for its ability to achieve simultaneous removal of organic soft tissue remnants as well as the mostly inorganic smear layer (McComb & Smith 1975, Goldman *et al.* 1982, Berg *et al.* 1986, Baumgartner & Mader 1987, Sen *et al.* 1995).

Ethylene diaminetetraacetic acid (EDTA) is a chelating agent for inorganic divalent cations including calcium ions forming soluble calcium chelates (Grossman 1988). It is known to aid in the removal of inorganic components of the smear layer formed on the root canal walls, in addition to its bactericidal effect due to the susceptibility to disinfectants and antibiotics brought about by chelating cations from the outer membrane of bacteria (Russell *et al.* 1999). Consequently, the currently recognized effective irrigation regime to accomplish smear-free canals comprises a high volume final flush of the root canal with 17% EDTA followed by NaOCl (Goldman *et al.* 1982, Yamada *et al.* 1983, Baumgartner & Mader 1987).

Glyde$^{TM}$ File Prep (Dentsply Maillefer, Ballaigues, Switzerland) gel is a root canal conditioner consisting of EDTA and carbamide peroxide in a water-soluble base. According to the manufacturers, it is designed to be used in conjunction with root canal instrumentation supplementary to NaOCl irrigation. Besides the internal bleaching effect enhanced by oxidation of carbamide peroxide, this gel-like preparation is claimed to facilitate endodontic shaping and cleansing presumably due to the adjunctive effect of EDTA on the smear layer and effervescence through oxygen release from the carbamide peroxide theoretically allowing dentinal shavings and root canal debris to be readily removed.

The aim of this study was to evaluate the effectiveness of Glyde$^{TM}$ File Prep used in conjunction with NaOCl irrigation in the removal of smear layer produced during root canal instrumentation.

## Materials and methods

### Sample preparation

Thirty-nine extracted mature human teeth with single canals of curvature less than 30° and with no previous root canal treatment were collected and stored in 10% formo-saline. These teeth were included based on uniform root length of approximately 16 mm measured from the lowest level of the CEJ to the root apices, as well as the uniform root and canal width based on radiographic measurement in the bucco-lingual and mesio-distal dimensions and the subsequent initial apical file size of size 20. Adherent soft tissue was mechanically removed without damaging the root surface. Inspection was further carried out to ensure there were no cracks, fractures or areas of root resorption. The teeth were distributed into 13 sets of three teeth of the same tooth type with each tooth randomly assigned to one of the three different irrigation regimes. Each group was then stored in a sealed container containing 0.9% saline, incubated at 37°C and maintained at 100% humidity between each experimental stage.

### Root canal chemo-mechanical instrumentation

For all teeth, coronal access cavity preparation was carried out in a conventional manner and the working length established 1 mm from the major apical foramen. Root canal preparation was completed using a step-back technique with K-Flex files (Kerr/Sybron, Romulus, MI, USA) commencing with an initial apical file of size 20 to a master apical file of size 60. Cervical step-back flare to file size 100 was carried out to allow accommodation of a fine finger spreader (Kerr/Sybron) to within 2 mm of the working length. Root canal irrigation was carried out with a 25-gauge needle placed at the cervical third of the canal length according to the three regimes described below and thereafter dried with sterile absorbent paper points and the access cavity sealed with cotton pellet and Cavit (ESPE, Seefeld, Germany).

*Group A (positive control)*: Routine irrigation with 0.5 mL of 1% NaOCl (Milton solution, Proctor & Gamble Ltd, Cincinnati, OH, USA) after each file and an additional final irrigation of 10 mL NaOCl at the completion of instrumentation.

*Group B (negative control)*: Routine irrigation with 0.5 mL of 1% NaOCl after each file and an additional final gradual irrigation of 10 mL of 17% EDTA (Pulpdent, Watertown, USA) at the completion of instrumentation.

*Group C (experimental group)*: Routine irrigation with 0.5 mL of 1% NaOCl after each file coated with Glyde$^{TM}$ File Prep and an additional final irrigation of 10 mL NaOCl at the completion of instrumentation.

### Light microscopic evaluation

After storage for 24 h, the teeth were grooved vertically with carborundum discs on the buccal and lingual surfaces under high power suction. These were then cleaned and dried prior to being split longitudinally with a chisel and a mallet into two halves.

Ten sets of the samples were designated for light microscopic evaluation. Both halves of each split tooth were divided into cervical, middle and apical thirds measured from the cemento-enamel junction to the terminus of the apical preparation, using an indelible pencil. All sections were stained with 1% methylene blue (David Bull Laboratories, Warwick, UK) for 2 min prior to being rinsed with 0.9% saline and left to bench dry. The stained sections were then attached to individual glass slides using beading wax, coded and randomly arranged for blind evaluation.

Scoring for the presence of smear layer for both halves of the split teeth was carried out visually for the cervical, middle and apical thirds by two independent examiners using a light microscope (Nikon Microscope SE, Nippon Kogaku KK, Tokyo, Japan) at ×40 and ×100 magnifications, according to the rating scale from 1–3 with scale 1 indicating a clean canal with no or very little debris present; scale 2 for debris present in less than half the evaluated canal region and scale 3 for debris occupying more than half the evaluated canal region.

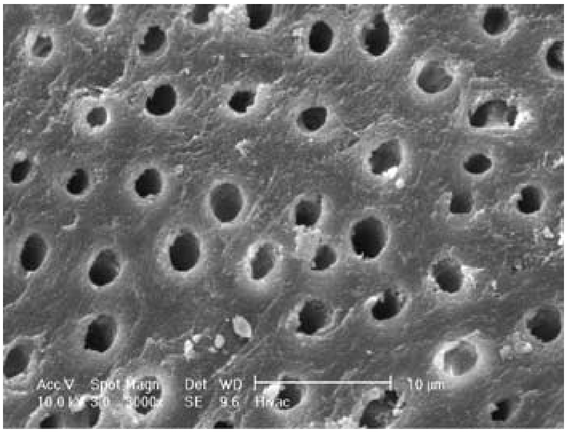

**Figure 1** SEM photomicrographs (×3000) of sample specimens with score 0 (Rome *et al.* 1985) showing no smear layer with opened dentinal tubules free of debris.

### Scanning electron microscopic evaluation

The remaining three sets of teeth were technically prepared for evaluation using scanning electron microscopy (SEM) (XL 30 SEM, Philips, FEI Company, Einhoven, the Netherlands). The sectioned halves were stored in 3% gluteraldehyde at 4°C prior to SEM processing. The SEM photomicrographs of the topography of the canal walls at three levels, namely the cervical, middle and apical thirds, were then taken in the middle of each region where the cross-section of the dentinal tubules were captured. The evaluation was carried out without the knowledge of specimen at ×1000 and ×3000 magnifications for the presence or absence of smear layer by two independent evaluators. Qualitative analysis of the canal cleanliness was based on the rating system developed by Rome *et al.* (1985): score 0 = no smear layer with opened dentinal tubules free of debris (Fig. 1); score 1 = smear layer present only in the apertures of the dentinal tubules (Fig. 2); score 2 = thin smear layer covering the root canal surface and dentinal tubular apertures (Fig. 3) and score 3 = heavy smear layer masking dentinal tubular apertures (Fig. 4).

Root canal cleanliness at the respective cervical, middle and apical third levels among the three groups was compared using analysis of variance (ANOVA) with posthoc Tukey HSD, while the cleanliness at different canal levels (cervical, middle and apical thirds) was compared within the same group using the repeated measurements of ANOVA with Bonferroni adjustments for multiple comparison. The level of significance was set at 5%.

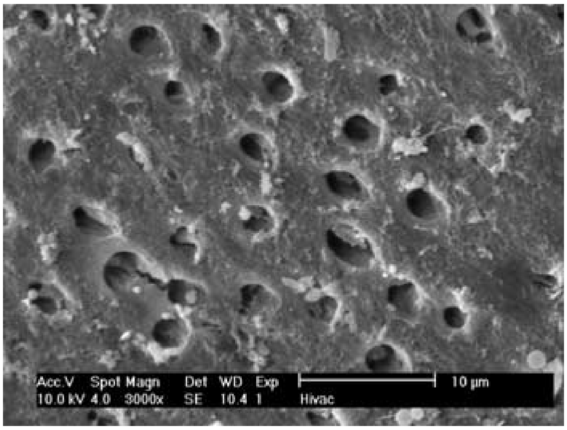

**Figure 2** SEM photomicrographs (×3000) of sample specimens with score 1 (Rome *et al.* 1985) showing smear layer present only in the apertures of the dentinal tubules.

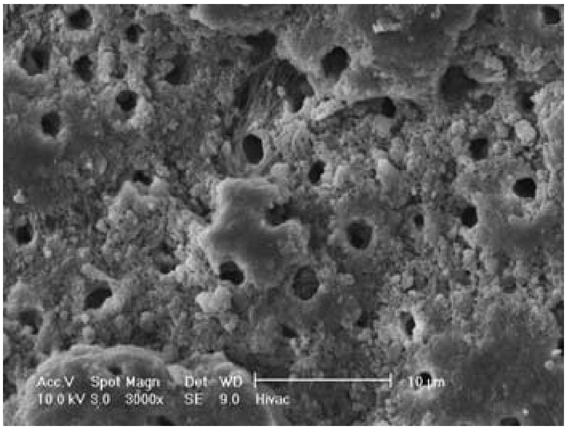

**Figure 3** SEM photomicrographs (×3000) of sample specimens with score 2 (Rome *et al.* 1985) showing thin smear layer covering the root canal surface and dentinal tubular apertures.

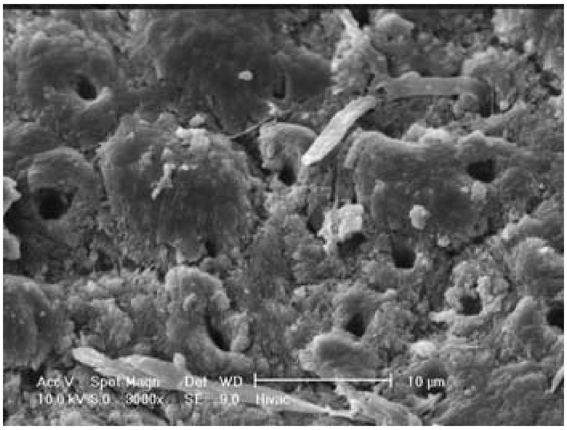

**Figure 4** SEM photomicrographs (×3000) of sample specimens with score 3 (Rome *et al.* 1985) showing heavy smear layer masking dentinal tubular apertures.

## Results

The debris scores in the cervical, middle and apical third levels among the three groups at different magnifications are presented in Tables 1–4. The results of the light microscopic evaluation at magnifications ×40 (Table 1) and ×100 (Table 2) were consistent showing no significant differences between the degree of cleanliness at different canal levels within each group, though there was a trend of a higher debris score at the apical third. In addition, it was observed that the degree of cleanliness between groups B (EDTA) and C (Glyde) was not significantly different at each canal level. However, groups B and C were significantly cleaner than group A using only NaOCl both at magnifications ×40 ($P = 0.000$ and $<0.014$, respectively; Table 1) and ×100 ($P < 0.001$; Table 2).

Under the SEM evaluation both at the higher magnifications of ×1000 and ×3000 (Tables 3 and 4), it was demonstrated that at the cervical and middle canal levels, there was significantly cleaner appearance in group B compared to group A ($P = 0.000$ and $<0.001$), as well as group C compared to the respective levels in group A ($P < 0.006$ and $<0.001$). However, the difference was not consistent at the apical levels. Similarly, there was also no significant difference in the canal cleanliness between the apical levels of groups B and C.

## Discussion

This study evaluated the effectiveness of smear layer removal associated with the use of Glyde$^{TM}$ File Prep in conjunction with 1% NaOCl irrigation during root canal instrumentation (group C) compared to two other

**Table 1** Cleanliness score at ×40 magnification (light microscope)

| Tooth | Level | Mean score | Standard error | Intertooth[a] Vs. | Level | *P*-value | Intratooth[b] Vs. | Level | *P*-value |
|---|---|---|---|---|---|---|---|---|---|
| A | Cervical | 2.700 | 0.100 | B | Cervical | 0.000 | A | Middle | 0.580 |
| | Middle | 2.550 | 0.090 | B | Middle | 0.000 | A | Apical | 0.230 |
| | Apical | 2.750 | 0.076 | B | Apical | 0.000 | A | Cervical | 1.000 |
| B | Cervical | 1.600 | 0.100 | C | Cervical | 0.834 | B | Middle | 1.000 |
| | Middle | 1.600 | 0.111 | C | Middle | 0.857 | B | Apical | 0.808 |
| | Apical | 1.800 | 0.145 | C | Apical | 0.096 | B | Cervical | 1.000 |
| C | Cervical | 1.700 | 0.105 | A | Cervical | 0.000 | C | Middle | 1.000 |
| | Middle | 1.700 | 0.125 | A | Middle | 0.000 | C | Apical | 0.074 |
| | Apical | 2.200 | 0.145 | A | Apical | 0.014 | C | Cervical | 0.150 |

[a] Tukey HSD. Based on observed means. The mean difference is significant at 0.05 level. [b] Bonferroni multiple comparisons, adjustment for multiple comparisons. Based on estimated marginal means. The mean difference is significant at 0.05 level.

**Table 2** Cleanliness score at ×100 magnification (light microscope)

| Tooth | Level | Mean score | Standard error | Intertooth[a] | | | Intratooth[b] | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Vs. | Level | *P*-value | Vs. | Level | *P*-value |
| A | Cervical | 2.650 | 0.076 | B | Cervical | 0.000 | A | Middle | 1.000 |
| | Middle | 2.600 | 0.082 | B | Middle | 0.000 | A | Apical | 0.230 |
| | Apical | 2.800 | 0.100 | B | Apical | 0.001 | A | Cervical | 0.920 |
| B | Cervical | 1.650 | 0.130 | C | Cervical | 0.699 | B | Middle | 1.000 |
| | Middle | 1.750 | 0.112 | C | Middle | 0.967 | B | Apical | 0.153 |
| | Apical | 2.050 | 0.138 | C | Apical | 1.000 | B | Cervical | 0.209 |
| C | Cervical | 1.800 | 0.100 | A | Cervical | 0.000 | C | Middle | 1.000 |
| | Middle | 1.800 | 0.111 | A | Middle | 0.001 | C | Apical | 0.439 |
| | Apical | 2.050 | 0.083 | A | Apical | 0.001 | C | Cervical | 0.390 |

[a]Tukey HSD. Based on observed means. The mean difference is significant at 0.05 level. [b]Bonferroni multiple comparisons, adjustment for multiple comparisons. Based on estimated marginal means. The mean difference is significant at 0.05 level.

**Table 3** Cleanliness score at ×1000 magnification (SEM)

| Tooth | Level | Mean score | Standard error | Intertooth[a] | | | Intratooth[b] | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Vs. | Level | *P*-value | Vs. | Level | *P*-value |
| A | Cervical | 2.000 | 0.000 | B | Cervical | 0.000 | A | Middle | – |
| | Middle | 2.000 | 0.000 | B | Middle | $<$0.001 | A | Apical | – |
| | Apical | 2.000 | 0.000 | B | Apical | 0.632 | A | Cervical | – |
| B | Cervical | 0.250 | 0.250 | C | Cervical | 0.024 | B | Middle | 1.000 |
| | Middle | 0.000 | 0.000 | C | Middle | $<$0.001 | B | Apical | 0.018 |
| | Apical | 1.750 | 0.250 | C | Apical | 0.220 | B | Cervical | 0.042 |
| C | Cervical | 1.000 | 0.000 | A | Cervical | 0.006 | C | Middle | – |
| | Middle | 1.000 | 0.000 | A | Middle | $<$0.001 | C | Apical | 1.000 |
| | Apical | 1.250 | 0.250 | A | Apical | 0.066 | C | Cervical | 1.000 |

[a]Tukey HSD. Based on observed means. The mean difference is significant at 0.05 level. [b]Bonferroni multiple comparisons, adjustment for multiple comparisons. Based on estimated marginal means. The mean difference is significant at 0.05 level.

**Table 4** Cleanliness score at ×3000 magnification (SEM)

| Tooth | Level | Mean score | Standard error | Intertooth[a] | | | Intratooth[b] | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Vs. | Level | *P*-value | Vs. | Level | *P*-value |
| A | Cervical | 2.250 | 0.250 | B | Cervical | 0.000 | A | Middle | 1.000 |
| | Middle | 2.000 | 0.000 | B | Middle | $<$0.001 | A | Apical | 0.173 |
| | Apical | 2.750 | 0.250 | B | Apical | 0.003 | A | Cervical | 0.545 |
| B | Cervical | 0.000 | 0.000 | C | Cervical | 1.000 | B | Middle | – |
| | Middle | 0.000 | 0.000 | C | Middle | $<$0.001 | B | Apical | 0.046 |
| | Apical | 1.250 | 0.250 | C | Apical | 0.632 | B | Cervical | 0.046 |
| C | Cervical | 0.000 | 0.000 | A | Cervical | 0.000 | C | Middle | $<$0.001 |
| | Middle | 1.000 | 0.000 | A | Middle | $<$0.001 | C | Apical | – |
| | Apical | 1.000 | 0.000 | A | Apical | 0.001 | C | Cervical | $<$0.001 |

[a]Tukey HSD. Based on observed means. The mean difference is significant at 0.05 level. [b]Bonferroni multiple comparisons, adjustment for multiple comparisons. Based on estimated marginal means. The mean difference is significant at 0.05 level.

regimes using NaOCl irrigation alone (group A) and NaOCl irrigation with EDTA as a final flush (group B). For optimal comparison of the efficiency of different irrigation regimes, measures were taken in the inclusion criteria and the study designed to ensure standardized canal size and canal curvature and the extent of canal enlargement, as well as the volume of irrigant delivered using a conventional needle. Although it was not the intention of the current study to compare the two evaluation methods using light and scanning electron microscopy, the combined evaluation results were assumed to provide more comprehensive information on the degree of canal cleanliness than when only one approach was used. Two magnifications were chosen for each evaluation method as a simple means to determine the consistency of the results of the evaluation. Light microscopic evaluation at lower magnifications of ×40 and ×100 allowed a general overview of the canal appearance. The limitation on the precise detectability of smear layer within the dentinal tubules could be supplemented by the clearer assessment of the intratubular packing of smear layer (Aktener *et al.* 1989) at high magnifications of ×1000 and ×3000 under SEM. In addition, the combined evaluations could also preclude the possible misleading interpretation if specific nonrepresentative areas were chosen for evaluation.

The results demonstrated that the conventional irrigation with NaOCl alone (group A) was comparatively ineffective in achieving thorough debridement of the root canal system (Tables 1–4). This corroborates with earlier studies where dentine shavings and/or smear layer were found to be still present after root canal instrumentation with NaOCl irrigation (Baker *et al.* 1975, McComb & Smith 1975, Goldman *et al.* 1981). On the other hand, in line with other studies (Yamada *et al.* 1983, White *et al.* 1984, Baumgartner & Mader 1987, Cengiz *et al.* 1990), the results from group B showed that 1% NaOCl irrigation during instrumentation and a final flush of 17% EDTA was significantly more effective in removing the instrumentation debris and smear layer from the root canal walls compared to group A.

Under light microscopic evaluation both at the magnifications of ×40 and ×100 (Tables 1 and 2), Glyde$^{TM}$ File Prep used in conjunction with NaOCl irrigation (group C) was significantly more effective in removing the smear layer than in group A where NaOCl alone was used; canal cleanliness was not significantly different at any level compared to group B using NaOCl and EDTA. In the study, Glyde$^{TM}$ File Prep was applied to the files and instrumentation was completed throughout the entire root canal length. This would theoretically facilitate intimate tissue–chemical contact (Andersen *et al.* 1992) between the canal wall and the EDTA within the Glyde$^{TM}$ File Prep with the anticipated improved efficacy of smear layer removal and hence cleaner canals compared to the EDTA group (group B), where irrigation was done by placement of irrigation needles slightly below the canal orifices. However, contrary to the expected outcome, the canal appearances from group C were not significantly cleaner than that of group B. It might be that the effervescence produced from the reaction between carbamide peroxide within Glyde$^{TM}$ File Prep and NaOCl resulted in bubbles occupying the already limited space available as well as preventing fresh NaOCl from reaching the apical canals by their coronal physical movement (Senia *et al.* 1971).

The apical canal region has been shown to be difficult to clean and this was also illustrated in our study (Table 1–4). Under SEM evaluations at both magnifications of ×1000 and ×3000 (Tables 3 and 4), the debris scores for group B, where 17% EDTA was used as final flush, was found to be similar to group C in which Glyde$^{TM}$ File Prep was used. This observation could be attributed to the comparatively smaller apical canal dimension hindering the penetration of the root canal irrigants resulting in limited contact with the root canal irrigant/conditioner (Ciucchi *et al.* 1989, Takeda *et al.* 1999).

Similarly, studies should be directed at investigation of smear layer removal with the use of Glyde$^{TM}$ File Prep supplementing NaOCl irrigation activated by ultrasonic delivery system. Ultrasonic irrigation has been shown to produce smear-free root canal surfaces (Cameron 1983, 1987) probably due to acoustic streaming provided direct physical contact of the ultrasonic file with the canal walls were avoided (Ahmad *et al.* 1987). Less debris and smear layer were observed in the apical regions of the canals than the coronal aspects as the magnitude and velocity of the acoustic streaming were demonstrated to be most intense at the apical regions of the ultrasonic file.

In addition, Baumgartner & Mader (1987) have reported that the combination of NaOCl and EDTA caused a progressive dissolution of the peritubular and intertubular areas, opening up the diameters of tubular orifices on the instrumented canal walls to 2.5–4.0 μm. Whether the EDTA within the Glyde$^{TM}$ File Prep would chelate calcium ions from the dentine, especially the hydroxyapatite-rich peritubular dentin, thereby softening the dentinal tissue and, thus, affecting the root canal dentin hardness (Madison & Krell 1984), remains a question that needs further investigation.

## Conclusions

Under the present experimental design, it was concluded that the effectiveness of smear layer removal from the instrumented canals by Glyde$^{TM}$ File Prep was not significantly different from that occurring with final flush of 10 mL 17% EDTA. Both irrigating regimes produced cleaner root canals than the groups irrigated with sodium hypochlorite solution alone. Root canal irrigation with 1% sodium hypochlorite alone did not remove smear layer effectively from the instrumented canals.

## Acknowledgements

This study was supported in part by a grant from the Year 2000 Undergraduate Research Opportunity Programme, National University of Singapore. The authors appreciate the statistical analysis provided by Mr. Dong Fang, National University of Medical Institute, National University of Singapore, Singapore. Technical assistance from Dr Li Zhimei is hereby acknowledged.

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